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Complete List of Authors:	Schmierer, Klaus; Queen Mary University of London, Blizard Institute (Neuroscience) Miquel, Marc; Barts Health NHS Trust, Clinical Physics
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Magnetic resonance imaging correlates of neuro-axonal pathology in the MS spinal cord

Schmierer K[1,2], Miquel ME[3]

[1]Queen Mary University of London, Barts and The London School of Medicine & Dentistry, Blizard Institute (Neuroscience), London, UK

[2]Barts Health NHS Trust, Clinical Board Medicine (Neuroscience), The Royal London Hospital, London, UK

[3]Barts Health NHS Trust, Clinical Physics, London, UK

Correspondence to: Klaus Schmierer, The Blizard Institute (Neuroscience), Queen Mary University of London, 4 Newark Street, London E1 2AT, UK, Tel: +44 02078826246, E-mail: k.schmierer@qmul.ac.uk

Short title: MRI of spinal cord axonal loss in MS

Key words: Multiple sclerosis, MRI, axonal loss, spinal cord, white matter, grey matter, diffusion, magnetisation transfer, cross sectional area, MR microscopy

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Abstract

In people with multiple sclerosis MS, the spinal cord is the structure most commonly affected by clinically detectable pathology at presentation, and a key part of the central nervous system involved in chronic disease deterioration. Indices, such as the spinal cord cross-sectional area at the level C_2 have been developed as tools to predict future disability, and - by inference - axonal loss. However, this and other histo-pathological correlates of spinal cord magnetic resonance imaging (MRI) changes in MS remain incompletely understood. In recent years, there has been a surge of interest in developing quantitative MRI tools to measure specific tissue features, including axonal density, myelin content, neurite density and orientation, among others, with an emphasis on the spinal cord. Quantitative MRI techniques including T_1 and T_2, magnetisation transfer and a number of diffusion-derived indices have all been applied to MS spinal cord. Particularly diffusion-based MRI techniques combined with microscopic resolution achievable using high magnetic field scanners enable a new level of anatomical detail and quantification of indices that are clinically meaningful.

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Introduction

In multiple sclerosis (MS), it is the spinal cord that is most commonly affected by clinically detectable pathology at presentation (Mowry *et al.*, 2009; Katz Sand, 2015). Lesions in the spinal cord suggestive of demyelination have been shown to predict a definitive diagnosis of MS in patients with radiologically isolated, as well as clinically isolated, syndromes (Okuda *et al.*, 2011) (Arrambide *et al.*, 2017), and more severe disability (Brownlee *et al.*, 2017) (Arrambide *et al.*, 2017). And as MS evolves over time, much of the permanent and deteriorating disability in people with MS affects lower body functions, including lack of sphincter control, sexual dysfunction, and impaired leg movement and coordination, resembling the clinical syndrome of progressive myelopathy (Giovannoni *et al.*, 2017; Kremenchutzky *et al.*, 2006; McDonald and Compston, 2006; Kearney *et al.*, 2015).

It is therefore not surprising that the pathological manifestations of MS in the spinal cord have attracted renewed interest, including attempts to better visualise and quantify histological changes non-invasively using magnetic resonance imaging (MRI). Combining MRI with (quantitative) histology enables investigation of fundamental associations between tissue features with clinical relevance, such as the grey and white matter (Schlaeger *et al.*, 2014), and the cell types affected by disease, including the degree of tissue loss measured using volumetric MRI (Losseff *et al.*, 1996), and the microscopic changes underlying this loss. Evidence suggests results obtained using brain samples cannot be directly translated to the spinal cord (McDowell *et al.*, 2014).

In this paper, we will review the pathological features of MS currently detectable on MRI of the spinal cord, with an emphasis on neuro-axonal loss, and on studies correlating MRI with histology. Recent pathological findings spell the need for further research into the spinal cord network and its destruction by MS. This work builds and expands on a recent topical review (Schmierer *et al.*, 2018).

MS lesions in the spinal cord

After it had been recognized by the early 1980s that MRI exceeds the sensitivity of computed tomography not only of the brain (Young *et al.*, 1981) but also of the spinal cord in detecting parenchymal lesions (Earnest *et al.*, 1985), the first study directly correlating T_2

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weighted (T_2W) MRI with MS lesions was published in 1994 when the case of a woman who died of MS at the age of 37 was reported, and imaging appearance correlated with histology (Nagao *et al.*, 1994). A series of 59 spinal cord samples from 19 cases of MS and three controls were subsequently investigated using proton density (PD) weighted MRI at two different field strengths (1 and 4.7 Tesla) (Nijeholt *et al.*, 2001). Correlation of MRI with histology in a proportion of the cases examined confirmed excellent visual match between lesions detected using histology and MRI at either field strength. Importantly, scans acquired at 4.7T additionally revealed a distinction between clearly demarcated lesions and rather diffuse changes, suggesting Wallerian (or retrograde) degeneration as a result of axonal transection in lesions (Trapp *et al.*, 1998) (Nijeholt *et al.*, 2001) (Dziedzic *et al.*, 2010).

Gilmore and co-workers were the first to shift the focus of correlative MRI-pathology studies on lesions affecting the spinal cord grey matter (Gilmore *et al.*, 2009/1). Using a 4.7T scanner, PD MRI was acquired in cord samples of 11 pwMS and two controls. Following MRI acquisition, samples were dissected and immuno-stained for myelin basic protein. N= 40 'white matter only' lesions, 55 mixed (white/grey matter) lesions, and one 'grey matter only' lesion were detected on PD MRI. Separating white and grey matter proportions of mixed lesions, 87% of histologically confirmed areas of white matter, and 73% of grey matter demyelination were detected using MRI, i.e. significantly more than in the neocortex (Geurts *et al.*, 2011), where partial volume effects, among others, adversely affect their detection (Gilmore *et al.*, 2009/1; Schmierer *et al.*, 2010).

Axonal loss in lesions and beyond

Significant axonal loss takes place in the MS spinal cord, degree of which appears most strongly associated with the duration of the disease. A recent study reported reduction of axonal density in the cortico-spinal tracts by 57–62% across all cord levels after a mean disease duration of 29 years (Petrova *et al.*, 2018), confirming earlier studies using tissue from pwMS with similar disease duration (Bjartmar *et al.*, 2000; Tallantyre *et al.*, 2009), whilst studies of material with shorter disease duration reported less pronounced axonal loss (Ganter *et al.*, 1999; DeLuca *et al.*, 2004). In line with this observation, axonal loss (be it within or beyond the margins of MS lesions (Bjartmar *et al.*, 2000; Dziedzic *et al.*, 2010)) is considered a major contributor to the relentless accrual of disability in pwMS over time.

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Separating the effects on MRI indices of inflammation and demyelination on the one, and axonal damage and loss on the other hand, remains challenging. Whilst in 2001 Nijeholt and co-workers highlighted the close relationship between areas of high signal on T_2W MRI with the extent of demyelination (Nijeholt *et al.*, 2001), a subsequent study by the same group described considerable T_2W MRI signal abnormalities in cord tissue not affected by lesions (non-lesional cord tissue) yet significant - and seemingly lesion-independent - axonal loss (Bergers *et al.*, 2002). Of note, the authors did not control for remote effects on axonal loss of lesions along the pathway examined, which have been shown to be of importance when examining the relationship between inflammation, demyelination and axonal loss (Petrova *et al.*, 2018).

Magnetisation transfer

Given the nonspecific nature of PD and T_2 weighted MRI, a range of quantitative MR techniques including magnetisation transfer (MT) (Lema *et al.*, 2017), diffusion (Cohen *et al.*, 2017) (Stikov *et al.*, 2015) and spectroscopic metabolite concentration (albeit not in *post mortem* samples) have been used to try and improve detection and quantification of microstructural changes in the MS spinal cord (Gass *et al.*, 2015). MT is a process by which macromolecular protons (for example, in myelin bi-phospholipid layers) and water protons (for example, in cerebro-spinal fluid) exchange magnetisation when exposed to an external magnetic field and a radio frequency saturation pulse. Changes in MT can be quantified and enable inferences about the underlying macromolecular content and structure (Tozer *et al.*, 2003).

A number of MT indices has been explored in *post mortem* MS brain (van Waesberghe *et al.*, 1999; Barkhof *et al.*, 2003; Schmierer *et al.*, 2004, 2007), where they were shown to be primarily associated with myelin, though inflammation, oedema (Vavasour *et al.*, 2011) and - particularly in NLSC - axonal loss (Petzold *et al.*, 2011) also contribute. The strong association of MT, as well as T_1 and T_2 relaxation times, with myelin was confirmed in a study by Bot and co-workers on cervical spinal cord (Bot *et al.*, 2004); and similar results were reported by Mottershead and co-workers in their study of spinal cord specimens employing a small bore high magnetic field (7T) scanner (Mottershead *et al.*, 2003). The

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latter study also highlighted an important issue when trying to separate MRI indices for axonal density and myelin content: that these tissue features themselves are quite strongly correlated (here, r= 0.67, p<0.0001) (Mottershead *et al.*, 2003).

Diffusion

The strong association between changes in myelin and axons in a demyelinating disease like MS was also an important challenge for experiments using diffusion MRI. Work in animal models suggested the assessment of the directionality of diffusion (axial, radial) might enable more reliable non-invasive quantification of myelin versus axonal damage and loss in the CNS (Song *et al.*, 2005). However, the apparently clear separation in the model proved difficult to reproduce in the human disease MS. Klawiter and coworkers applied DTI to *post mortem* spinal cord from nine pwMS and five control subjects using a 4.7T system (Klawiter *et al.*, 2011). They placed regions of interest in areas semi-quantitatively graded as normally myelinated, mildly (<50%) and moderate-severely (>50%) demyelinated. Increasing radial diffusion (D_{rad}) values were associated with the degree of demyelination but so was the extent of axonal loss, whilst axial diffusion (D_{ax}), radial diffusivity and relative anisotropy did not predict axonal density *in isolation*. Analysis of myelin and axonal count simultaneously indicated that both tissue features contributed independently to changes in radial diffusivity, relative anisotropy and MD (Klawiter *et al.*, 2011). The study by Mottershead and co-workers using a 7 Tesla MRI system also reported diffusion data. The 'diffusion standard deviation index' (SDI), a measure of anisotropy, was calculated after images had been acquired at two different diffusion gradient strengths. Moderate correlation emerged between the SDI and axonal count (r= 0.61, p<0.001) as well as myelin content (r= 0.51, p<0.001).

Since single diffusion tensor models did not reliably enable extraction of indices specific to axonal damage and loss, more complex set-ups have recently combined multiple diffusion tensors for this purpose, such as diffusion basis spectrum imaging (DBSI) (Wang *et al.*, 2011). DBSI models myelinated and unmyelinated axons as anisotropic diffusion tensors, and cells and oedema/extracellular space as isotropic diffusion tensors. Quantitative histological analysis of *post mortem* MS cervical spinal cord specimens (n=3) suggested that DBSI-determined indices of cellularity, axons and myelin acquired on a small bore 4.7T magnet

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are closely associated with those pathologies identified and quantified by conventional histology (Wang *et al.*, 2015).

Another promising diffusion-based attempt at increasing specificity for tissue components and their injury by MS is neurite orientation dispersion and density imaging (NODDI) (Jespersen *et al.*, 2012; Zhang *et al.*, 2012). An index of orientation dispersion is defined to characterize the angular variation of neurites. NODDI has been used both *in vivo* as well as for validation experiments on spinal cord samples including MS and control tissue (Grussu *et al.*, 2017), By et la. 2016). Strong correlation was detected between a quantitative histology index defined as "circular variance" (CV) and the NODDI derived variable "orientation dispersion index" (ODI), suggesting ODI may provide a non-invasive marker of CV (Grussu *et al.*, 2016). Comparison with more conventional DTI metrics such as mean diffusivity, fractional anisotropy, D_{ax} and D_{rad} suggest NODDI may indeed provide more precise estimates of the complexity of dendrites and axons (Grussu *et al.*, 2017).

The envelope of non-invasive visualisation and quantification of spinal cord pathology has recently been pushed further by successful acquisition of 3D anatomic image data (50 µm isotropic resolution) alongside 100 µm isotropic resolution diffusion data of an entire spinal cord (Calabrese *et al.*, 2018) (figure 1). This was made possible by a multi-segment acquisition lasting 280 h, and automated image segment composition. The ability to acquire such datasets provides a platform for spinal cord lesion detection, automated volumetric grey matter segmentation, and quantitative spinal cord morphometry including estimates of cross sectional dimensions and grey matter fraction throughout the length of the cord (figure 2) (Calabrese *et al.*, 2018).

The novel techniques outlined above, including high resolution MR microscopy (Calabrese *et al.*, 2018), DBSI (Wang *et al.*, 2011, 2015) and NODDI are likely to offer advantages in terms of tissue specificity which, in the case of NODDI, notably include indices to assess spinal cord grey matter (Zhang *et al.*, 2012; Grussu *et al.*, 2015, 2017). If preliminary reports can be confirmed, and reproducibility further improved (Grussu *et al.*, 2015; Tanguy Duval *et al.*, 2017), these techniques may offer significant steps in the quest for accurate *in vivo* assessment of spinal cord pathology in MS, perhaps in combination with other techniques,

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such as MT or multi-component relaxometry (Tanguy Duval *et al.*, 2017). It is encouraging that several initiatives to improve the standardisation of spinal cord MRI analysis have gone underway that will likely facilitate MRI-pathology studies yet further thereby enabling more rapid validation of new techniques in the future (Grussu, n.d.; De Leener *et al.*, 2017).

Spinal cord cross-sectional area as a proxy of axonal loss?

Whilst the quantification of tissue "microstructure" using quantitative MRI is of significant interest to potentially better understand the pathophysiology of MS in the spinal cord, none of the above mentioned techniques have entered the realm of clinical trials, let alone clinical practice, where the detection of lesions using conventional MRI techniques continues to dominate. However, limitations in (i) the association between demyelinating lesions and axonal loss and (ii) lesion detection in the MS spinal cord due to technical artefacts, have highlighted the need for alternative indices with potential to be robust predictors of axonal damage and loss.

Since the seminal study by Losseff and coworkers (Losseff *et al.*, 1996) more than 20 years ago, numerous clinical studies underpinned the correlation between a reduction of the spinal cord cross-sectional area (CSA) and disability (Losseff *et al.*, 1996; Kearney *et al.*, 2015; Aymerich *et al.*, 2018). CSA loss has also been applied as an outcome in a small number of clinical trials (Kapoor *et al.*, 2010; Rice *et al.*, 2015), and various methods have been used to measure it including semi-automated edge finding (Lin *et al.*, 2003), edge detection with partial volume corrections (Tench et al. 2005), voxelwise mapping (Rocca *et al.*, 2013), an active surface model (Kearney *et al.*, 2014) and semi-automated cord volume estimation techniques (Lukas *et al.*, 2015).

Based on experimental data, CSA loss - and its association with clinical disease progression - has long been considered as a key substrate of axonal degeneration. However, recent data suggest the macro-/microscopic relationship between CSA and nerve fibre loss is not as straightforward (Petrova *et al.*, 2018).

Following preliminary work on a small number of specimens by Bjartmar and co-workers (Bjartmar *et al.*, 2000), a recent study comprehensively sampled spinal cords of 13 pwMS

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with a mean disease duration of 29 years, and five healthy controls to assess the association between axonal density and CSA . Using just under of 400 tissue blocks a reduction of the CSA of 19-24% was detected at all (cervical, thoracic and lumbar) levels with white and grey matter areas contributing equally across levels. However, compared to controls axonal density was reduced by 57-62%. And whilst disease duration was a predictor of reduced axonal density, CSA was not, and neither were separate indices of proportional grey or white matter area (Petrova *et al.*, 2018).

This surprising lack of correlation evidently challenges the concept of CSA shrinkage being a predictor of axonal loss, and other factors had to be considered, including "space filling" through gliosis, since this would be expected to counteract the area reducing effect of axonal loss (Bjartmar *et al.*, 2000; Hampton *et al.*, 2013). Since both grey and white matter contributed equally to the reduction of CSA, it is unlikely that long tract systems (cortico-spinal, dorsal ascending, and others) are exclusively contributing to the sum total CSA change. In line with this finding, it is has been suggested that neuronal shrinkage and loss, and a reduction in neurite orientation dispersion (Grussu *et al.*, 2017) may contribute to both disability and loss of CSA (Christopher P. Gilmore *et al.*, 2009/2; Schirmer *et al.*, 2009). Finally, based on synaptophysin immuno-staining, a substantial loss of synapses has recently been reported affecting both non-lesional and lesional cord grey matter. This loss was associated with grey matter area shrinkage (Petrova *et al.*, 2016). Some (or all) of these results may also explain that reported associations between CSA and disability have in a number of recent studies been rather moderate (Schlaeger *et al.*, 2014; Aymerich *et al.*, 2018).

Improving the non-invasive prediction of tissue changes in MS - is MR microscopy realistic?

The pathology of MS is complex, and its aetiology and pathogenesis on the microstructural level remain incompletely understood. It is, thus, not surprising that attempts at MRI quantification of specific tissue features (axons, myelination status, microglial activation, gliosis, etc) are challenging.

Until quite recently, key MRI-pathology studies of the spinal cord made hardly any reference to the grey matter which, similar to its importance in the brain (Carassiti *et al.*, 2018), is a

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likely key factor for the clinical manifestations of MS. Long spinal cord white matter tract systems, such as the cortico-spinal and the dorsal ascending (sensory) tract systems, with their largely longitudinal orientation are obviously ideal candidates to model new techniques including attempts at measuring the g-ratio in vivo (Stikov *et al.*, 2015; Campbell *et al.*, 2017; T. Duval *et al.*, 2017). However, when looking at MS as a disease, and the need for pwMS, their health care professionals and scientists to better understand and manage their condition, it is important to consider the spinal cord as a functional network with millions of perpendicular connections that are damaged in MS and impact on function (Bourane *et al.*, 2015; Grussu *et al.*, 2016; Petrova *et al.*, 2016).

Given the importance of immune-mediated demyelination for the degree of axonal loss throughout MS (Montalban *et al.*, 2017; Petrova *et al.*, 2018), there is an ongoing need for further improved techniques to detect lesions across the length of the spinal cord, to be used as outcomes in trials of new compounds for the clinical management of pwMS (Giovannoni *et al.*, 2015). This is particularly true against the backdrop of the poor prediction of disability based on lesions detected using conventional MRI techniques (Dekker *et al.*, 2018).

The recently developed toolboxes for both improved MRI and pathology measurement of spinal cord pathology provide exciting new opportunities to integrate the complexity of MS pathophysiology. The techniques used have come a long way including new standardised methods to map spinal cord MRI onto histology (and *vice versa*). In *post mortem* studies of the MS brain, this problem has been recognized for some time (Moore *et al.*, 2000), and various techniques were subsequently developed to improve registration, including use of a stereotaxic frame (Schmierer *et al.*, 2003; Bö *et al.*, 2004), imaging the unfixed brain *in situ* with subsequent rescanning of the fixed tissue and use of customised cutting panels (Bö *et al.*, 2004; Fisher *et al.*, 2007), and most recently, the introduction of individually manufactured cutting panels using 3D printing technology (Luciano *et al.*, 2016).

Compared to the brain the spinal cord appears like a less challenging structure to match MRI with histology. However, the recently published systematic framework for histological quantification (Grussu *et al.*, 2016) combined with landmark-guided co-registration and

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high-resolution imaging (Calabrese *et al.*, 2018) provide insights into how complex (and successful), new approaches to correlative MRI-pathology studies of *post mortem* spinal cord can be (Grussu *et al.*, 2017), against the backdrop of a much stronger emphasis on histological quantification (optical density indices, stereology, orientation dispersion, etc.) (Schmierer *et al.*, 2004; Grussu *et al.*, 2016; Carassiti *et al.*, 2018), over and above established qualitative indices (Bergers *et al.*, 2002).

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Disclosure

The authors declare no conflict of interest with respect to the contents of this paper.

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Figure legends

Figure 1:

Magnetic resonance imaging (MRI) of an entire human spinal cord at the level of the central canal using a 7 Tesla small bore MR system. Techniques used included T_2* weighted gradient echo (A) and diffusion-weighted MRI (B). The coloured image (C) represents a map of fractional anisotropy (directionality) derived from diffusion weighted imaging. Reproduced with permisson from Calabrese, et al. 2018.

Figure 2:

Multi-contrast axial magnetic resonance images of a human spinal cord at different levels. Contrasts include: T_2*, T_2* weighted gradient echo; B0, b=0 image from diffusion acquisition; DWI, isotropic diffusion weighted image; FA, fractional anisotropy; FAC, directionally colored fractional anisotropy. Reproduced with permisson from Calabrese, et al. 2018.

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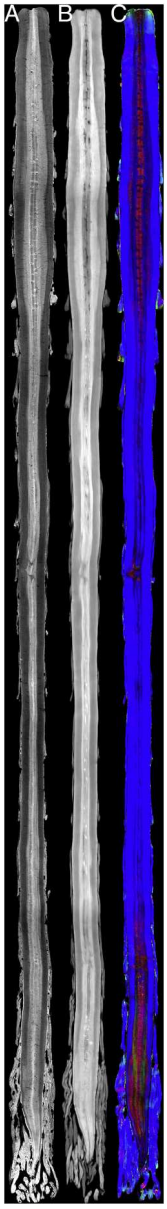

Figure 1

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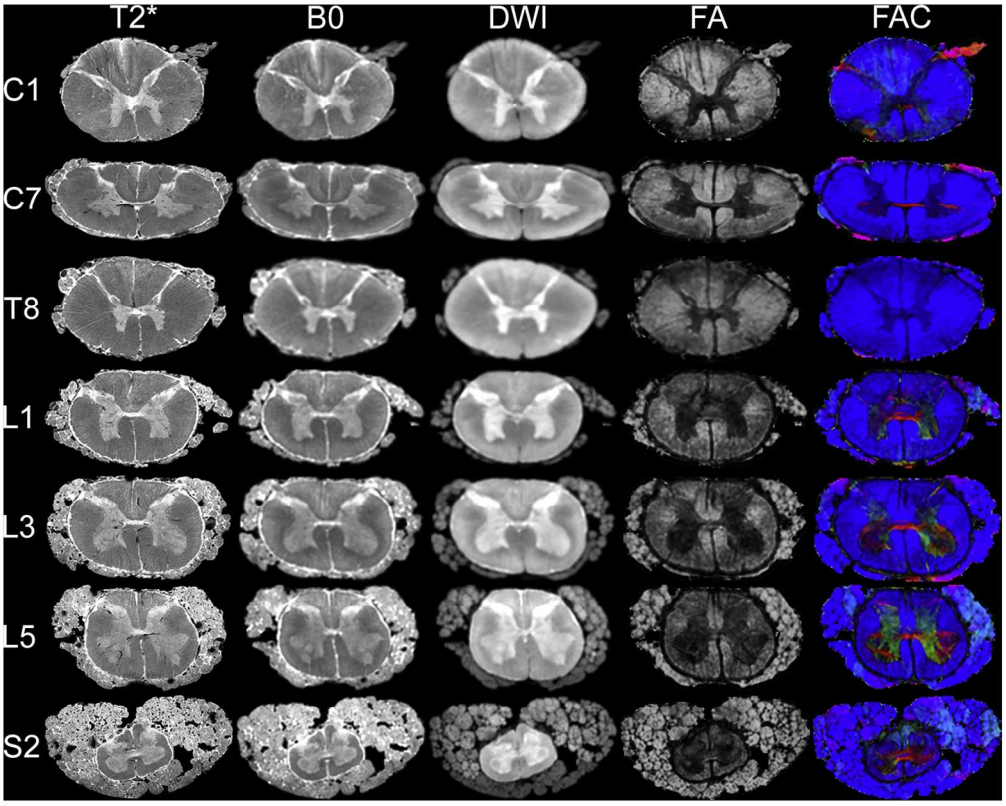

Figure 2

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